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spectra of seven isomeric hexen-1-ols have been determined in order to detect any correlations between fragmentation patterns and molecular structure. The double bond isomers have proved to be easily recognizable by their different base peaks. The mass spectra of the corresponding cis and trans isomers are in general very similar. Some differences between the spectra of the geometric isomers of 2- and 3-hexen-1-ols exist, however, which are sufficient for qualitative analysis. Gas chromatographic retention times for the isomeric hexen-1-ols are given.

Studies on the alkaloids of *Thalictrum Thunbergii* DC. XIV. Structures of thalicberine and O-methylthalicberine. (6). Eiichi Fujita, Toshiaki Tomimatsu and Yoko Kano. *Yakugaku Zasshi*, 83, 160 (1963) in Japanese.—In order to elucidate the structure of the substance III, derived from the basic decomposition product of ozone oxidation of O-methylthalicberine methylmethine (I), synthesis of 2-methoxy-4-methyl-5-ethylphenyl 2-methyl-3-ethyl-5,6-dimethoxyphenyl ether (II) was carried out. One of the starting materials, 3-bromo-4-methyl-5-ethyl-1,2-dimethoxybenzene (VIII), was synthesized from isovanillin (III) by its Clemmensen reduction to IV, introduction of acetyl group by the Friedel-Crafts reaction, Clemmensen reduction of its products to VI, bromination to VII, and finally by O-methylation to VIII. The other substance XVI was synthesized from *m*-cresol (IX) by its acetylation to X, Fries rearrangement followed by Clemmensen reduction to XII, and O-methylation to 3-methyl-4-ethylanisole (XIII), which was reduced and hydroxyl group introduced through diazotization. The position of this hydroxyl group was confirmed through the negative Gibbs reaction and identity of the infrared spectrum (in CHCl_3) of the O-methyl ether (XVII) of this substance with that of O-methyl ether (XVII) of VI synthesized by a known method. The Ullmann condensation of these 3-bromo-4-methyl-5-ethyl-1,2-dimethoxybenzene (VIII) and 2-methoxy-4-methyl-5-ethylphenol (XVI) gave the desired substance (II).

The alkaloids of American lotus, *Nelumbo Lutea*. S. M. Kupchan, B. Dasgupta, E. Fujita and M. L. King. *Tetrahedron*, 19, 227 (1963).—The leaves and stems of *Nelumbo lutea* (Willd.) Pers. from Wisconsin yielded the alkaloids nuciferine, (\pm)-armepavine, and two apparently new alkaloids to which we have assigned structures V ((-)-N-norarmepavine) and XII ((-)-N-nornuciferine).

Biochemistry

Biosynthesis of S-methyl-L-cysteine and S-methyl-L-cysteine sulfoxide from methionine in garlic. Michiyasu Sugii, Shigeharu Nagasawa and Tomoji Suzuki. *Chem. Pharm. Bull. Japan*, 11, 135 (1963).—An excised aerial part of garlic plant was cultivated in water containing L-methionine ^{35}S . After 24 hours' feeding the amino acid, a fraction was extracted from the plant and ^{35}S -labeled amino acid were separated by paper chromatography and Dowex 50 column. Methionine, methionine sulfoxide, S-methyl-L-cysteine and S-methyl-L-cysteine sulfoxide were obtained, but no other radioactive amino acids was detected in this experiment.

These facts show that the S-methyl group of S-methyl-L-cysteine and its sulfoxide is originated from methionine.

Isolation of (–) S-propenyl-L-cysteine from garlic. Michiyasu Sugii, Tomoji Suzuki and Shigeharu Nagasawa. *Chem. Pharm. Bull. Japan*, **11**, 548 (1963).—During the studies of amino acids containing sulfur and the related compound in garlic, the authors have isolate a new amino acid in crystalline state in leucine fraction on Dowex 50 column and confirmed that the crystals are (–)-S-propenyl-L-cysteine ($\text{CH}_3\text{-CH=CH-S-CH}_2\text{-CH(NH}_2\text{)-COOH}$). Five kg. of garlic bulb yielded 30 mg. of pure new amino acid, m. p. 195° (decomp.) $[\alpha]_{\text{D}}^{20} -15^\circ$, Rf. 0.80 (PhOH-0.08% NH_4OH =4:1) and (BuOH-AcOH- H_2O =4:1:2).

Studies on β -(Pyrazolyl-N)-L-alanine. Masazumi Takeshita, Yasutomi Nishizuka and Osamu Hayaishi. *J. Biol. Chem.*, **238**, 660 (1963).—1. In 1957 Shinano and Kaya isolated a new amino acid from the pressed juice of watermelon, the chemical structure of which was later proposed to be β -(pyrazolyl-N)-L-alanine by Noe and Fowden. The identity of the compound was confirmed in our laboratory by independent synthesis and by comparison of the natural amino acid with the L isomer obtained from the synthetic material. 2. Several properties of the new amino acid are described. 3. A new enzyme, pyrazolealaninase, was purified from cells of *Pseudomonas cruciviae*, grown on the new amino acid. The enzyme catalyzed the conversion of β -(pyrazolyl-N)-L-alanine to stoichiometric quantities of pyrazole, pyruvic acid, and ammonia. 4. The enzyme had its optimal pH at 8.6, and required pyridoxal phosphate as a coenzyme. 5. The enzyme was specific for β -(pyrazolyl-N)-L-alanine. It did not react with the D isomer. Other amino acids tested, including the D and L isomers of histidine, tryptophan, tyrosine, serine, and alanine, were totally inert.

Enzymic synthesis of niacin nucleotides from 3-hydroxyanthranilic acid in mamalian liver. Yasutomi Nishizuka and Osamu Hayaishi. *J. Biol. Chem.*, **238**, PC 483 (1963).

Adenosine diphosphate-dependent threonine dehydrase activity in extracts of *Clostridium tetanomorphum*. Osamu Hayaishi, Malcolm Geeter and Herbert Weissbach. *J. Biol. Chem.*, **238**, 2040 (1963).—Two threonine dehydrases have been separated by chromatography on diethylaminoethyl cellulose from extracts of *Clostridium tetanomorphum*. At low threonine concentrations a marked dependence on the presence of adenosine diphosphate is seen; this disappears as the substrate level increases. The nucleotide effect is quite specific, only guanosine diphosphate and inosine diphosphate showing some activity. At pH values below 8.0 the adenosine diphosphate requirement is accompanied by a yeast extract requirement. The latter factor has not been identified.

Enzwmic fromation of D-kynurenine. K. Higuchi, S. Kuno and O. Hayaishi. *Federation Proc.*, **22**, No. 2 (1963).—Rabbits fed D-tryptophan excrete D-kynurenine in the urine (*J. Biochem., Japan*, **25**, 71, 1937). The present work was undertaken

to study the site of D-kynurenine formation in the animal and the enzyme reactions involved. A dialyzed, soluble fraction of a rabbit ileum homogenate, methylene blue (4×10^{-5} M), adenosine (1×10^{-3} M), air, and D-tryptophan (6×10^{-3} M) appear necessary for D-kynurenine formation. The addition of a DPNH-generating system markedly stimulates the reaction. The enzyme appears to be different from L-tryptophan pyrrolase (oxygenase). The pH optimum is about 8.0 in 0.1 M Tris buffer. Approximately 3 umoles of D-kynurenine are formed per hour with 6-11 mg of crude enzyme protein under optimal conditions. The stoichiometry indicate the consumption of one mole each of D-tryptophan and O_2 to yield equivalent amounts of D-kynurenine and formate. D-Kynurenine was identified by its UV absorption spectrum and the D-configuration was assigned on the basis of chromatography by the procedure of Price and Dodge (*J. Biol. Chem.*, **223** 449 1956).

Crystallization and some properties of metapyrocatechase. Mitsuhiro Nozaki, Hiroyasu Kagamiyama and Osamu Hayaishi. *Biochem. Biophys. Res. Comm.*, **11**, 65 (1963).

Metabolic fate of kynurenic acid- C^{14} intraperitoneally administered to animals. Takashi Murachi, Kinji Tsukada and Osamu Hayaishi. *Biochem.*, **2**, 304 (1963).— Kynurenic acid-2- or 3- C^{14} was intraperitoneally administered to the rat, mouse, hamster, rabbit, guinea pig, cat and dog in a dose of $0.6 \mu\text{mole}$ per 100 g body weight. With all the animals tested, more than 90 % of the dose given was excreted in the urine during the first 24 hours, with little or no radioactivity in expired CO_2 or carcass. Most of the radioactivity of the first-day urine was present as unchanged kynurenic acid (80-100%). The excretion of small amounts of quinaldic acid (1.3-4.8%) and quinaldylglycine (0.3-1.8%) was also detected.

S-Alkylcysteinase: Enzymatic cleavage of S-methyl-L-cysteine and its sulfoxide. Junichi Nomura, Yasutomi Nishizuka and Osamu Hayaishi. *J. Biol. Chem.*, **238**, 1441 (1963).— 1. A new enzyme, S-alkylcysteinase, was isolated from S-methyl-L-cysteine-adapted cells of *Pseudomonas cruciviae*. The enzyme was purified approximately 30-fold by protamine treatment, ammonium sulfate fractionation, calcium phosphate gel adsorption, and chromatography on a column of diethylaminoethyl cellulose. 2. The enzyme catalyzed the stoichiometric conversion of S-methyl-L-cysteine to methyl mercaptan, pyruvic acid, and ammonia, and of S-methyl-L-cysteine sulfoxide to methyl methanethiolsulfinic acid, pyruvic acid, and ammonia. 3. The pH optimum of the enzyme is 8.8, and pyridoxal phosphate is required as a coenzyme. 4. In addition to S-methyl-L-cysteine and its sulfoxide, various other S-alkyl-L-cysteines, S-alkyl-L-cysteine sulfoxides, and S-(carboxyalkyl)-L-cysteines could serve as efficient substrates. Both methionine and cysteine were inert as substrates.

Quinolinic acid: A precursor to nicotinamide adenine dinucleotide. Anthony J. Andreoli, Masayuki Ikeda, Yasutomi Nishizuka and Osamu Hayaishi. *Biochem. Biophys. Res. Comm.*, **12**, 92 (1963).

Quinolate transphosphoribosylase: A mechanism of niacin ribonucleotide formation from quinolinic acid. Shigenobu Nakamura, Masayuki Ikeda, Hiroshi Tsuji, Yasutomi Nishizuka and Osamu Hayaishi. *Biochem. Biophys. Res. Comm.*, **13**, 285 (1963).

Metapyrocatechase. I. Purification, crystallization and some properties. Mitsuhiro Nozaki, Hiroyuki Kagamiyama and Osamu Hayaishi. *Biochem. Z.*, **338**, 582 (1963).—The purification and crystallization procedure of the oxygenase, metapyrocatechase, from *Pseudomonas arvilla* is described. The main steps of the procedure consist of 1. preparation of crude extract by sonic oscillation, 2. deoxyribonuclease treatment, 3. acetone fractionation, 4. chromatography on DEAE-cellulose and 5. crystallization by ammonium sulfate. The enzyme was found to be protected almost completely from inactivation by air by the presence of a low concentration of an organic solvent such as acetone or ethanol. An overall purification of about 30 fold was achieved. The crystalline enzyme was completely homogeneous as judged by ultracentrifugation and its molecular weight was estimated to be approximately 140,000. Certain other properties of the crystalline enzyme are described.

Energy-linked regulation of threonine metabolism by ADP. Masanobu Tokushige, H. R. Whiteley and Osamu Hayaishi. *Biochem. Biophys. Res. Comm.*, **13**, 380 (1963).

Studies on the metabolism of kynurenic acid. III. Enzymatic formation of 7, 8-dihydroxykynurenic acid from kynurenic acid. Hiroshi Taniuchi and Osamu Hayaishi. *J. Biol. Chem.*, **238**, 283 (1963).—1. A partially purified enzyme preparation from *Pseudomonas fluorescens* (ATCC 11299B) adapted to tryptophan catalyzes the formation of a new compound from kynurenic acid with the consumption of equimolar amounts of reduced nicotinamide adenine dinucleotide or reduced nicotinamide adenine dinucleotide phosphate and oxygen. This enzyme was referred to as kynurenic acid hydroxylase. 2. The intermediate compound was isolated in a pure form but has not been crystallized, since it decomposes to 8-hydroxykynurenic acid upon drying. Available evidences indicate that it is identical with 7,8-dihydrokynurenic acid-7,8-diol. 3. This compound was dehydrogenated by a partially purified enzyme preparation to form 7,8-dihydroxykynurenic acid. NAD but not NADP was the essential cofactor. This enzyme was referred to as 7,8-dihydrokynurenic acid-7,8-diol dehydrogenase. 4. 7,8-Dihydroxykynurenic acid was isolated, identified, and established as an intermediate in the metabolism of kynurenic acid. 5. The mechanism of formation of 7,8-dihydroxykynurenic acid from kynurenic acid is discussed in connection with the biological origin of catechol and the mechanism of enzymic hydroxylation.

Allosteric proteins. Osamu Hayaishi. "Seibutsu-butsuri," **3**, 70 (1963), in Japanese.—Review article.

The preparative and pharmacological studies of levo and dextro 9-aza-des-N-morphinan (2, 3, 4, 4a-tetrahydro-1H, 6H-5, 10b-propanophenanthridin-9-ol). Goro

Hayashi, Mikio Takeda, Hiroshi Kugita, Norio Sugimoto and Hajime Fujimura. *Chem. Pharm. Bull., (Tokyo)*, **11**, 489 (1963).—See, this Bulletin, **41**, 224 (1963).

Syntheses of 5-(aminoacylamido)-3-methylisoxazole derivatives and their analgesic action. Shojiro Uyeo, Hajime Fujimura and Atsushi Asai. *Yakugku Zasshi*, **83**, 195 (1963), in Japanese.—See, this Bulletin, **41**, 225 (1963).

Syntheses of 5-(aminoacylamido)-3-methylisoxazole derivatives and their analgesic action. II. Shojiro Uyeo, Hajime Fujimura and Atsushi Asai. *Yakugku Zasshi*, **83**, 198 (1963), in Japanese.—See, this Bulletin, **41**, 225 (1963).